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苍白秤钩风的炔酸成分*

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摘要 从苍白秤钩风（*Diploclisia glaucesens*）的茎干中分离到1个炔酸，9－炔－十八酸，其结构通过波谱和化学的方法得到鉴定。经筛选具有PAI－1活性。同时还得到三十碳酸，十一碳酸和β－谷甾醇。

关键词 苍白秤钩风，防己科，9－炔－十八酸，PAI－1活性

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A Acetylenic Acid from *Diploclisia glaucescens*

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Abstract A acetylenic acid, ocetadecanoic acid－9－yne has been isolated from the stem of *Diploclisia glaucescens*. Its structure was elucidated by spectral method and chemical reaction. In addition, triacontanoic acid, undecanoic acid and β－sitosterol were also obtained. One of them, ocetadecanoic acid－9－yne was isolated from *D. glaucescens* for the first time. It shows stronger PAI－1 bioactivity.

Key words *Diploclisia glaucescens*, Menispermaceae, Ocetadecanoic acid－9－yne, PAI－1 Bio－activity

The genus *Diploclisia* of Menispermaceae include *Diploclisia affinis* and *Diploclisia glaucescens* (Institute of Botany, Academia Sinica, 1980). *Diploclisia glaucescens* (Bl.) Diels is a creeping plant growing in the south and southeast of Yunnan province. The leaves have been used in the treatment of rheumatism, venereal disease and poisoning, snake venom etc. (Jingsu New Medical College *et al*, 1977). In Sri Lanka, the leaves are also used to treat biliousnes (Miller *et al*, 1985).

There are some chemical studies on *D. glaucescens*. The major components are phytoecdysteroids, which were isolated from the roots (Virbala *et al*, 1989), stems (Bandara *et al*, 1989a), and seeds (Miller *et al*, 1985). Especially, the stem afforded high yield of ecdysterone , which is over 3% (Bandara *et al*, 1989a). Other phytoecdysteroids are 20－hydroxyecdysone, makisterone

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A, 24 (28) – dehydromakisterone A, 24 – *epi* – makisterone A and pterosterone (Miller *et al*, 1985). From the stems of this plant a bidesmosidic triterpenoidsaponin, diploclisin and three triterpenoids: serjanic acid, phytilaccagenic acid and 3 – O – β – D – glucopyranosyl phytilaccagenic acid (Bandara *et al*, 1989b) are also isolated. Jayasinghe *et al* (1993) isolated vibo – quercitol, four new saponins (Ⅰ – Ⅳ) and alkaloid, stepharine (Jayasinghe *et al*, 1992) from the stems.

A preliminary survey (TLC test) has not indicated the presence of ecdysterone in the plant growing in Yunnan. We only isolated four compounds from the liposoluble fraction of the roots of *D. glaucescens*. This paper deals with the isolation and structure elucidation of four compounds.

RESULTS AND DISCUSSION

The $CHCl_3$ soluble fraction of ethanolic extract from the stems of *D. glaucescens* was purified by chromatographed on a silica gel column to give four compounds (1 – 4).

Compound 1 white solids. Its molecular formula $C_{18}H_{32}O_2$ was determined by FAB – MS spectrum, in which the $[M+1]^+$ ion peak at 281 was exhibited. The infrared (IR) spectrum showed absorption due to hydroxyl group at 3500 ~ 2500 cm^{-1}, a methylene group at 2924 and 2857 cm^{-1}, and a $C=O$ group at 1712 cm^{-1}. The $C\equiv C$ stretch is a weak band occurring at 2200 cm^{-1}. The ^{13}C NMR spectra showed a carbonyl carbon (δ179.69), two acetylenic carbons (δ80.07, 80.36), 14 methylene carbons (δ33.99 ~ 22.65) and 1 methyl carbon (δ14. 04), which was the signal of terminal methyl carbon of a straight aliphatic chain. The ^1H NMR spectrum and the two – dimensional HMBC indicated that methyl protons at δ0.85 (3H, t) could be assigned to H – 18. The methylene protons (δ2.32, t, J = 7.5Hz) bearing the carbonyl carbon, which was coupled to two methylene proton signal at δ1.62. The methylene (δ2.11, t, J = 6.4Hz) adjacent to an acetylene was coupled to the δ1.42 methylene. Comparison of the spectral date with ocetadecanoic acid, compound 1 was identified as acetylenic acid (Shen, 1988).

On oxidation of 1 with permanganate, compound 1 cleavage in mixtures of carboxylic acids (Andrew, 1980). Then, these carboxylic acids were acetylated with dimethyl – sulfuric and analyzed by GC/ MS.

$$CH_3(CH_2)_7C\equiv C(CH_2)_7COOH \xrightarrow{aq.KMnO_4} CH_3(CH_2)_7COOH + HOOC(CH_2)_7COOH$$

The GS – MS analysis indicated that presence methyl nonanoate and dimethyl sulfate. So the acetylenic bond was assigned to C – 9 position. Thus, the structure of compound 1 was established as ocetadecanoic acid – 9 – yne.

Compounds 2, 3 and 4 were identified as triacontanoic acid, undecanoic acid and β – sitosterol respectively, by comparison of their ^1H, ^{13}C NMR and MS spectral date with those of authentic sample.

EXPERIMENT

General Procedures The melting points were determined by XRC - 1 microwarm melt - point-meter. All melting points uncorrected. ^1H and ^{13}C NMR and 2D NMR spectra were recorded in $CDCl_3$ with Bruker AM - 400MHz instrument, using TMS as an internal standard. EIMS: 70eV, FAB - MS spectra were measured with VG Auto - Spec 3000 spectrometer. IR spectra were measured on Bio - Rad FTS 135 instrument, in KBr pellets. GC - MS were measured with finigan - 4510. CC was carried out with silica gel. TLC: precoated silica gel G (Marine Chemical Industry Factory, Qingdao). The spots were visualized by spraying with 20% H_2SO_4 followed by heating.

Plant material *D. glaucescens* was collected at Xishuanbanna, Yunnan, China. Specimen was taxonomically identified by Mr. Wang Hong and deposited in the Herbarium of Kunming Institute of Botany, the Chinese Academy of Sciences.

Extraction and separation The dried and powdered stems of *D. glaucescens* (3.6 kg) were extracted with 95% EtOH for three times. After removal of EtOH in *vacuo*, a brown residue (108 g) was remained, which was successively fractionated with $CHCl_3$ and CH_3OH. The $CHCl_3$ fraction was dissolved into 5% HOAc solution. The chloroform layer was concentrated in *vacuo* to give viscous residue (46 g), which was repeatedly chromatographed on silica gel column, eluting with $CHCl_3$ - MeOH to give fraction A ~ G.

Fraction B (15.6 g) was subjected to CC over silica gel eluting with petroleum ether - acetone (98:2 ~ 80:20), gave three compounds 1 (56 mg), 2 (100 mg), and 3 (30 mg).

Fraction C (7.7 g) was chromatographied on silica gel column eluting with petroleum ether - acetone (95:5 ~ 80:20) to give compound 4 (30 mg) by recrystallization in acetone.

Compound 1 (0.0016%), a white solid, $C_{18}H_{32}O_2$, $IR\nu_{max}^{KBr}$ cm^{-1}: 2500 ~ 3500, 2930, 2857, 2617, 2200, 1712. FAB - MS m/z (%): 281 $[M+1]^+$ (74), 263 (45), 239 (39), 67 (65). ^1H NMR ($CDCl_3$): δ 2.32 (2H, t, J = 7.5Hz, H - 2), 1.62 (2H, m, H - 3), 2.11 (2H, t, J = 6.4Hz, H - 8), 1.42 (2H, m, H - 7), 0.85 (3H, t, H - 18). ^{13}C NMR ($CDCl_3$): δ 179.69 (C - 1), 33.99 (C - 2), 24.66 (C - 3), 28.76 (C - 4), 29.22 (C - 5), 29.43 (C - 6), 28.98 (C - 7), 18.77 (C - 8), 80.01 (C - 9), 80.36 (C - 10), 18.77 (C - 11), 28.98 (C - 12), 29.67 (C - 13), 29.11 (C - 14), 28.89 (C - 15), 31.66 (C - 16), 22.65 (C - 17), 14.04 (C - 18).

Oxidation of 1 **with permanganate** The oxidation of Compound 1 (1 mg) with 1% aq. permanganate for 1 h at 70℃. The reaction mixture was extracted with diethyl ether. The diethyl ether layer was heated with 3 drops dimethyl - sulfuric acid for 0.5 hr at ambient temperature. After acetylated, added 2 mL water, allowed to stand a while, then extracted with diethyl ether. The diethyl ether layer was analyzed by Finigan - 4510. The analysis indicated the major components were dimethyl sulfate and methyl nonanoate.

Triacontanoic acid (2) (0.0028%), $C_{30}H_{60}O_2$, a white solid, EI - MS (70eV) m/z: 452

$[M]^+$, 434 $[M-H_2O]^+$. The data of ^1H, ^{13}C NMR, IR and MS were identical with that of authentic sample.

undecanoic acid (3) (0.0009%), white solid, $C_{11}H_{22}O_2$, mp 72 ℃. MS (20eV) m/z: 186 $[M]^+$, ^1H NMR ($CDCl_3$): δ 3.61 (2H, t, CH_2OH), 0.85 (3H, t, J = 6.6Hz), 1.60 (2H, m), 1.23 (14H, m), 2.32 (2H, t, J = 7.4Hz), ^{13}C NMR ($CDCl_3$): δ179.2 (C-1), 34.02 (C-2), 24.22 (C-3), 29.09, 29.24, 29.35, 29.43, 29.68, 31.04, 22.68, 14.04.

β-sitosterol (4) (0.0009%), colorless needless (acetone), $C_{29}H_{50}O$, mp 139 ~ 140℃, EI-MS (70eV) m/z: 414 $[M]^+$. It was identified by mixed melting point, and comparison of spectral data with those of authentic sample.

Bioactivity testing of ocetadecanoic acid-9-yne (1) showed stronger PAI-1 bioactivity.

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